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# BIOSYNTHESIS OF FATTY ACIDS BY SUBCELLULAR FRACTIONS OF NEWBORN MOUSE SKIN*

DAVID I. WILKINSON, PH.D.

## ABSTRACT

Homogenates of newborn mouse skin were centrifuged at 900 × *g*, 15,000 × *g*, and 100,000 × *g*. Sediment from the last two centrifugations, as well as the 100,000 × *g* supernatant fraction incorporated [1-$^{14}$C]-acetyl CoA and [1,3-$^{14}$C]-malonyl CoA into fatty acids which were analyzed by chromatographic procedures and scintillation counting. All three fractions, especially the supernatant fraction, utilized malonyl CoA in labeling saturated acids. The particulate fractions used both malonyl CoA and acetyl CoA in labeling unsaturated acids. Several cofactor requirements were established. Coenzyme A was markedly inhibitory. When oxygen was replaced by nitrogen, incorporation of both precursors was increased by 50%

Comparison of the incorporation of [1-$^{14}$C]-acetyl CoA into fatty acids of the 15,000 × *g* and 100,000 × *g* pellet fractions was studied with and without oxygen. Results showed that chain elongation predominated as the mode of labeling of unsaturated acids. This was confirmed by estimating the extent of carboxyl carbon labeling. The principal chain extensions were: 16:1 → 18:1 → 20:1, 18:2 → 20:2, and 20:4 → 22:4. Desaturase activity was not observed, even in the 100,000 × *g* (microsomal) pellet fraction.

The distribution of radioactivity among polyunsaturated acids of whole skin after exposure to [$^{14}$C]-acetate was compared to the pattern observed with a 15,000 × *g* pellet fraction after incubation with [$^{14}$C]-acetyl CoA. Results indicated that 18:2ω6 (linoleic acid), 20:3ω6, and others were probably labeled in whole skin by a retroconversion mechanism which was inoperative under the conditions used with the subcellular fractions.

The biosynthesis of fatty acids in skin has been studied by the incorporation of labeled acetate into saturated and unsaturated acids by whole skin in vitro [1–3]. The observed pattern of radioactivity among individual fatty acids represents the combined contributions of various biosynthetic reactions, which may be carried out by different subcellular fractions. These reactions have been studied in detail in other tissues. The principal reactions are: de novo synthesis, chain elongation of existing fatty acids by 2-carbon units, and desaturation. De novo synthesis is usually carried out by cytoplasmic enzymes, desaturation by microsomal enzymes, and chain elongation by both mitochondrial and microsomal enzymes.

These reactions have not been studied in detail in skin, although Aso has described de novo synthesis and chain elongation in guinea-pig epidermis [4,5]. The present study was undertaken to obtain estimates of the contributions of various subcellular fractions to overall fatty acid biosynthesis, with special reference to the formation of polyunsaturated fatty acids, which are elaborated in other tissues by chain elongation followed or preceded by the desaturation of preformed fatty acids.

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* From the Department of Dermatology, Stanford University School of Medicine, Stanford, California 94305.

## MATERIALS AND METHODS

### *Tissue Preparation*

Newborn Swiss-Webster mice, 1–2 days old, were sacrificed and their skins removed, scraped free from adhering fat, weighed, rinsed in buffer, and homogenized in ice-cold buffer containing potassium phosphate, 100 mM (pH 7.4), reduced glutathione (GSH), 1.5 mM, sucrose, 250 mM, and EDTA, 1 mM, using a glass Duall homogenizer and 3 ml buffer /gm skin. The homogenate from 10 gm of skin was centrifuged (900 × *g*, 15 min, 4°C), and the pellet resuspended in fresh buffer (15 ml) and recentrifuged. The combined supernatant fractions were centrifuged (15,000 × *g*, 30 min, 4°C), and the resulting supernatant fraction was centrifuged at 100,000 × *g* for 90 min in a Spinco model L centrifuge. The high-speed supernatant fraction (HSS) was retained, and the pellets from the last two centrifugations were resuspended in phosphate buffer, pH 7.4, containing GSH, 1.5 mM. Protein was determined on aliquots by the Lowry method, and adjusted to concentrations of about 10 mg/ml.

Because rigorous purification of the 15,000 × *g* (mitochondrial) and 100,000 × *g* (microsomal) fractions was not attempted, and because the degree of cross-contamination of each was not assessed by suitable enzyme determinations, these fractions are referred to by their centrifugal designation in this study.

### *Radioisotopes*

[1,3-$^{14}$C]-Malonyl CoA (spec act 19.8 mCi/mM) and [1-$^{14}$C]-acetyl CoA (spec act 53 mCi/mM), both >97% radiopurity were obtained from New England Nuclear. Sodium [1-$^{14}$C]-acetate (spec act 53 mCi/mM) was obtained from Schwarz-Mann.

*Cofactors*

The source and concentration of the following reagents in the final mixtures were as follows unless indicated otherwise. Obtained from Sigma: NADH (grade III, 98%), 0.75 mM; NADPH (type I, 95–99%), 0.75 mM; $MnCl_2$, 5 mM; nicotinamide, 3 mM; pyridoxal 5'-phosphate, 0.4 mM; DL-isocitric acid, 2 mM; malonyl CoA, 0.1 $\mu$M. Obtained from Nutritional Biochemicals: ATP (99%), 5 mM; coenzyme A (96%), 0.2 mM; acetyl CoA, 0.1 $\mu$M. Obtained from J. T. Baker: $MgCl_2$, 5 mM; $NaHCO_3$, 20 mM.

*Incubation*

Experimental mixtures contained subcellular protein (5 mg/ml unless specified), cofactors and substrates in a total volume of 1 ml in capped culture tubes gassed with $O_2$ or $N_2$. These were heated at 37°C for 30 min in a shaking waterbath. Reactions were terminated by addition of $CHCl_3$—$CH_3OH$ or a KOH pellet as described below.

In one experiment, portions of each of a number of skins were pooled and minced with scissors. Subcellular fractions were prepared from the remaining skin. The minced skin (100 mg) was incubated for 2 hr in Krebs-Ringer buffer (3 ml) at 37°C with sodium [1-$^{14}$C]-acetate (2.5 $\mu$Ci/ml) in $O_2$ with shaking. The skin was homogenized in $CHCl_3$—$CH_3OH$, and the polyunsaturated fatty acids isolated by techniques described below.

*Extraction and Chromatography*

Reaction mixtures were usually extracted with $CHCl_3$—$CH_3OH$ (20 ml, 2:1, v/v with 0.005% 2,6-di-t-butyl-p-cresol) and the extracts shaken with 0.37 % KCl solution (4 ml). Lower phases were taken to dryness in a rotary evaporator. The residues were methylated, and methyl esters purified and resolved according to degree of unsaturation on $Ag^+$-impregnated thin-layer plates. These techniques have been described previously [3,6]. Bands corresponding to saturated, monounsaturated, and polyunsaturated methyl esters were scraped off, extracted with $CH_2Cl_2$ (containing 20% $CH_3OH$ in the case of polyunsaturates), and extracts evaporated to dryness. The residues were redissolved in scintillation counting medium (toluene containing PPO, 4.0 gm/l and POPOP, 0.05 gm/l) and their radioactivity assayed.

In other experiments, bands were redissolved in petroleum ether after recovery from the thin-layer plates, the solutions reduced in volume to about 50 $\mu$l, and methyl esters resolved by gas-liquid chromatography (GLC), using a 10% EGSS-X polyester stationary phase in a column maintained at 170°C for saturated and monoene samples, and 180°C for polyunsaturated. Effluent from the column was split in a 2:1 ratio, the smaller flow leading to a flame ionization detector at 260°C and the greater flow to a Packard 852 fraction collector where the methyl esters were condensed at 1-min intervals in glass tubes fitted with cellulose filters. These tubes with filters were placed in scintillation vials and their radioactivity assayed.

Identification of GLC peaks and linearity of detector response (peak area) were studied by injection of known amounts of methyl esters of saturated (obtained from Sigma, and Applied Science Labs) and unsaturated acids (from Nu Chek Prep, Elysian, Minn.). The detector/collector split ratio was obtained by carrier gas flow measurement with the oven at operating temperature, and efficiency of collection calculated by injection of aliquots of methyl [1-$^{14}$C]-stearate and [1-$^{14}$C]-linoleate in hexane.

In some experiments, in order to measure radioactivity incorporated into total fatty acids, reactions were stopped by addition of a KOH pellet, the tubes flushed with nitrogen, capped, and heated at 70°C for 60 min, diluted with water (3 ml), and extracted with hexane (2 × 2 ml). These extracts were discarded. The aqueous phase was reextracted with hexane–ether (2:1 v/v; 2 × 5 ml) after acidification (2 N HCl). These extracts were washed with water, dried ($Na_2SO_4$), evaporated, and the residue dissolved in toluene scintillation medium and counted.

Radioactivity was measured with a Beckman LS-100 liquid scintillation counter at 88% efficiency for $^{14}$C.

*Carboxyl labeling.* A 15,000 × *g* subcellular fraction was incubated as described in the legend to Table IV, and its fatty acids isolated as described above and recovered from the GLC fraction collector tubes by rinsing with hexane. The counts associated with the carboxyl carbon atom were measured by methods already published [6], which were applied to several acids possessing most of the radioactivity.

## RESULTS

### *Substrate Preference*

The HSS, and the 15,000 × *g* and 100,000 × *g* particulate fractions incorporated [$^{14}$C]-labeled precursors into saturated and unsaturated fatty acids. Using the specific activity of each precursor, radioactivity data were converted to picomoles of incorporated substrate (Table I). With malonyl CoA, radioactivity appeared mainly, and in the

TABLE I

*Incorporation of [$^{14}$C]-precursors into fatty acids of subcellular fractions*

| Fraction | Fatty acid class | Labeled precursor | | |
|---|---|---|---|---|
| | | [1,3-$^{14}$C]-malonyl CoA | [1-$^{14}$C]-acetyl CoA | [1-$^{14}$C]-acetate |
| 15,000 × *g* pellet | saturates | 1091 | 237 | 17 |
| | monoenes | 91 | 293 | 15 |
| | polyenes | 95 | 302 | 14 |
| 100,000 × *g* pellet | saturates | 1941 | 78 | 9 |
| | monoenes | 137 | 39 | 5 |
| | polyenes | 132 | 31 | 2 |
| HSS | saturates | 6943 | 16 | 2 |
| | monoenes | 19 | 7 | — |
| | polyenes | — | — | — |

Results are expressed as picomoles of precursor incorporated/mg protein/0.5 hr (mean values of 2 experiments). Mixtures contained ATP, NADH, NADPH, niacinamide, isocitrate, $MgCl_2$, CoA, $KHCO_3$, protein (5 mg), and [1,3-$^{14}$C]-malonyl CoA (0.5 $\mu$Ci), [1-$^{14}$C]-acetyl CoA (0.5 $\mu$Ci), or [1-$^{14}$C]-acetate (2.5 $\mu$Ci) in 1 ml volume in $O_2$. Unlabeled malonyl CoA (0.1 $\mu$M) was included with [$^{14}$C]-malonyl CoA, and acetyl CoA (0.1 $\mu$M) with [$^{14}$C]-acetyl CoA. Values from the 2 experiments did not differ from each other by more than 11% of the mean value in any instance.

case of HSS exclusively, in saturated fatty acids. Acetyl CoA was utilized extensively in the labeling of both saturated and unsaturated acids by the particulate fractions but not by HSS. Acetate was relatively little used by all fractions.

*Cofactor Requirements*

Cofactor requirements for incorporation of malonyl and acetyl CoA by the 15,000 × *g* and 100,000 × *g* pellet fractions are shown in Table II. Malonyl CoA required ATP, NADPH, but not NADH; acetyl CoA required ATP, NADH, and NADPH. No absolute requirement for divalent cations was observed; $Mn^{++}$ was strongly inhibitory for acetyl CoA. Incorporation was favored generally by anaerobic conditions. The strongest inhibition was caused by coenzyme A and was concentration-dependent (Fig. 1) above 0.05 mM.

Further experiments were performed using [1-$^{14}$C]-acetyl CoA. Incorporation was linear with time up to about 30 min (Fig. 2). The 15,000 × *g* pellet material incorporated 4–6 times as much labeled precursor into fatty acids as the 100,000 × *g* pellet (Tables I, III; the lower values in Table I are due to the presence of CoA). In both cases omission of oxygen increased radioactivity by about 50% in saturated and unsaturated fatty acids.

*Labeling of Fatty Acids*

Individual fatty acids were isolated by TLC and GLC procedures in order to determine the mechanism of labeling by acetyl CoA.

Radioactivity associated with the principal fatty acids is shown for reaction mixtures with and without oxygen (Table IV). As regards the relative magnitude of labeling, saturated fatty acids showed: 14:0 > 16:0 ≈ 18:0 ≫ 20:0, and monoenes showed: 20:1 > 18:1 ≫ 16:1 > 14:1. The chromatographic procedures used failed to detect radioactivity eluting from the GLC column ahead

TABLE II

*Cofactor requirements*

| Cofactor omitted | [1,3-$^{14}$C]-malonyl CoA | | [1-$^{14}$C]-acetyl CoA | |
|---|---|---|---|---|
| | 15,000 × *g* | 100,000 × *g* | 15,000 × *g* | 100,000 × *g* |
| NONE | 100 | 100 | 100 | 100 |
| ATP | 8 | 16 | 20 | 19 |
| NADH | 70 | 100 | 23 | 38 |
| NADPH | 37 | 35 | 48 | 25 |
| Niacinamide | 78 | 83 | 81 | 86 |
| $MgCl_2$ | 95 | 103 | 75 | 98 |
| Isocitrate | 62 | 115 | 75 | 123 |
| CoA | 192 | 280 | 260 | 220 |
| $MnCl_2$ | 75 | 120 | 140 | 210 |
| $NaHCO_3$ | 100 | 165 | 105 | 106 |
| Pyridoxal phosphate | 110 | 120 | 115 | 150 |
| *$O_2$ | 145 | 152 | 155 | 149 |

Relative extent of incorporation of [$^{14}$C]-precursors (0.05 μCi added) into total fatty acids of particulate fractions when each cofactor indicated was omitted. After incubation for 30 min, reactions were stopped by addition of a KOH pellet. Results are expressed as percentage of incorporation by the complete mixture, and are mean values from duplicate incubations, which did not differ from each other in any instance by more than 6% of the mean value.

* When $O_2$ was omitted, tubes were flushed with $N_2$.

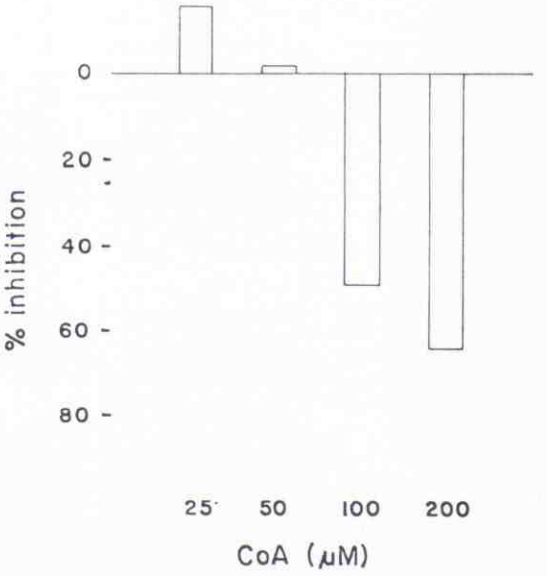


FIG. 1: Inhibition of incorporation of [1-$^{14}$C]-acetyl CoA into fatty acids of 15,000 × *g* fraction by coenzyme A. Other cofactors present are given in the legend to Table II. Results are mean values from duplicate incubations.

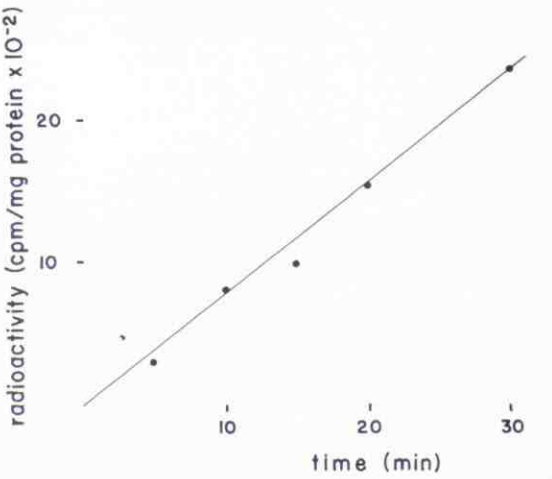


FIG. 2: Time-course of labeling with acetyl CoA. Reaction mixtures (duplicate) contained ATP, NADH, NADPH, isocitrate, acetyl CoA, [1-$^{14}$C]-acetyl CoA (0.05 μCi), and 2.5 mg each of 15,000 × *g* and 100,000 × *g* protein, with $N_2$.

TABLE III

*Effect of anaerobic conditions on [$^{14}$C]-acetyl CoA incorporation*

| Fatty acid class | 15,000 × *g* | | 100,000 × *g* | |
|---|---|---|---|---|
| | $O_2$ | $N_2$ | $O_2$ | $N_2$ |
| Saturates | 646 | 886 | 241 | 247 |
| Monoenes | 527 | 879 | 89 | 158 |
| Polyenes | 692 | 1037 | 69 | 154 |

Results are expressed as picomoles of acetyl CoA incorporated into fatty acids of particulate fractions/mg protein/0.5 hr. Mixtures contained ATP, NADH, NADPH, isocitrate, [1-$^{14}$C]-acetyl CoA (0.15 μCi), acetyl CoA, and protein. Values are means of 2 experiments and did not differ from each other by more than 10% of the mean value in any instance.

TABLE IV

*Incorporation of acetyl CoA into individual fatty acids*

| Fatty acid class | Particulate fraction | | | |
|---|---|---|---|---|
| | 15,000 × *g* | | 100,000 × *g* | |
| | $O_2$ | $N_2$ | $O_2$ | $N_2$ |
| Saturates | | | | |
| 14:0 | 296 | 395 | 123 | 130 |
| 16:0 | 178 | 275 | 25 | 29 |
| 18:0 | 171 | 279 | 15 | 20 |
| 20:0 | 16 | 36 | — | — |
| Monoenes | | | | |
| 14:1 | 28 | 37 | 2 | 4 |
| 16:1 | 47 | 52 | 6 | 13 |
| 18:1 | 213 | 331 | 22 | 43 |
| 20:1 | 363 | 650 | 41 | 77 |
| Polyenes (ω6) | | | | |
| 18:2 | 13 | 68 | 2 | 4 |
| 20:2 | 383 | 575 | 29 | 66 |
| 20:3 | 28 | 45 | 2 | 4 |
| 20:4 | 27 | 58 | 3 | 6 |
| 22:4 | 307 | 476 | 17 | 40 |

Results are expressed as in Table III. Mixtures (1.0 ml) contained ATP, NADH, isocitrate, [1-$^{14}$C]-acetyl CoA (0.5 μCi), acetyl CoA, and protein (10 mg). Fatty acids were separated by TLC and GLC.

of 14:0. Of the polyunsaturated acids, 20:2ω6† and 22:4ω6 possessed most of the radioactivity. Generally, incorporation was increased by about 50% when oxygen was replaced by nitrogen except in the case of the saturated fatty acids of the 100,000 × *g* fraction which were only slightly changed, but the distribution of radioactivity among individual saturated and unsaturated acids was unaffected.

These results indicated the presence of a chain elongation mechanism, but there was no evidence for desaturase activity.

† Specific isomers of polyunsaturated acids are identified by this system of notation. In this case, the last double bond is located 6 carbons from the methyl end of the molecule.

*Carboxyl Labeling*

For each of the fatty acids indicated, the radioactivity carried by the carboxyl carbon was expressed as a percentage of the total in the molecule (Table V). These values indicated that chain elongation by a single acetate unit was probably the exclusive mechanism of labeling of unsaturated fatty acids, and the predominant mechanism for saturated.

*Comparison of Polyunsaturated Fatty Acids Labeling in Whole Skin and Subcellular Fractions*

The data in Table VI are a comparison of the mass and radioactivity distribution of polyun-

TABLE V

*Labeling of carboxyl carbon*

| Fatty acid | % of $^{14}$C in carboxyl |
|---|---|
| 14:0 | 43 |
| 16:0 | 69 |
| 18:0 | 73 |
| 18:1 | 93 |
| 20:1 | 87 |
| 20:2ω6 | 97 |
| 22:4ω6 | 81 |

Percentage of radioactivity of individual fatty acids associated with carboxyl carbon. Experimental details are given in legend to Table IV using protein from a 15,000 × *g* fraction. Each fatty acid was recovered from GLC and treated as described in reference 6. Values are means of duplicate experiments, which did not differ from each other by more than 5% of the mean in any instance.

TABLE VI

*Distribution of mass and radioactivity of polyene fatty acids of whole skin and 15,000 × g pellet*

| Fatty acid | Mass | | Radioactivity | |
|---|---|---|---|---|
| | Whole skin | 15,000 × *g* | Whole skin | 15,000 × *g* |
| 18:2ω6 | 50.7 | 46.5 | 22.5 | 1.6 |
| 18:3ω6 | 2.0 | 1.8 | 7.7 | 0.3 |
| 18:3ω3 | 2.1 | 1.6 | 4.1 | — |
| 20:2ω6 | 3.2 | 2.2 | 10.1 | 47.8 |
| 20:3ω6 | 2.8 | 2.4 | 15.9 | 3.4 |
| 20:4ω6 | 20.5 | 27.9 | 2.1 | 3.3 |
| 20:5ω3 | 2.2 | 1.1 | 0.3 | 1.9 |
| 22:3ω6 | 1.1 | 1.1 | 1.1 | 1.6 |
| 22:4ω6 | 2.6 | 3.6 | 11.6 | 38.3 |
| 22:5ω6 | 1.9 | 1.3 | — | — |
| 22:5ω3 | 0.9 | 2.2 | — | — |
| 22:6ω3 | 1.6 | 7.6 | — | — |

Distribution of mass and radioactivity of polyene fatty acids of whole skin, after incubation of the tissue with [$^{14}$C]-acetate, and a 15,000 × g fraction after incubation as described in the legend of Table IV, using $O_2$. Results are expressed as % of total mass or radioactivity.

saturated acids obtained from whole skin after exposure of the tissue to [$^{14}$C]-acetate, and a 15,000 $\times$ $g$ subcellular fraction which had been incubated with [1-$^{14}$C]-acetyl CoA and cofactors as usual. Data for a 100,000 $\times$ $g$ fraction are omitted, because the patterns were similar except that incorporation was only about ¼ that of the 15,000 $\times$ $g$ fraction. As shown in Table VI, mass patterns were fairly similar except that the subcellular fraction had larger amounts of $C_{22}$ polyenes. Incorporation patterns were obviously different. The 15,000 $\times$ $g$ fraction showed most counts in 20:2ω6 and 22:4ω6 but few in 18:2ω6 (linoleic acid) and 20:3ω6. These results are discussed below.

## DISCUSSION

Differential centrifugation of epidermal homogenates has been described by Rosett et al [7] and by Bagatell et al [8]. Both groups prepared purified mitochondrial fractions and showed that epidermal mitochondria differed from classical rat liver mitochondria in morphology and enzymatic activity. The preparations used in the present study were derived from whole skin, and the particulate fractions therefore contain constituents from several skin compartments including epidermis. We have found that the elongation 20:4ω6 → 22:4ω6 occurs mostly in the dermis (unpublished data). It is therefore probable that the labeling of 22:4ω6 observed here (Table IV, VI) is due to the presence of dermal enzymes.

The incorporation of different precursors into fatty acids by the three subcellular fractions shown in Table I resembles that recorded for other systems. For instance, the HSS probably contains a fatty acid synthetase complex which readily utilizes malonyl CoA in de novo synthesis of saturated acids, similar to that found in the supernatant fractions of liver [9], brain [10], aorta [11], adipose tissue [12], and guinea-pig epidermis [4]. The utilization of acetyl CoA by HSS is lower than that of malonyl CoA by a factor of about 400. The HSS was not studied further as the principal interest lay with unsaturated acids.

The 15,000 $\times$ $g$ and 100,000 $\times$ $g$ pellet fractions utilized both acetyl CoA and malonyl CoA, but acetate only poorly. The mechanisms involved have been studied extensively in other systems. For instance, rat liver mitochondria are generally accepted as having two synthetic mechanisms, the predominant mechanism being chain elongation of preexisting fatty acids using acetyl CoA [9], and de novo synthesis via malonyl CoA proposed by Harlan and Wakil [14]. Mitochondria and microsomes of rat brain can use both precursors in chain elongation of saturated and unsaturated acids [15]. The data in Table I suggest that malonyl CoA is not very actively incorporated via chain extension as it appears mainly in saturated acids. However, further experiments are necessary before definite conclusions can be drawn.

The incorporation of both malonyl and acetyl CoA requires ATP, NADH, and NADPH (Table II). The 100,000 $\times$ $g$ fraction did not require NADH. Because the incorporation of malonyl CoA requires ATP, and that of acetyl CoA does not require $HCO_3^-$, synthesis by the de novo pathway must be present only to a minor extent, as this route uses malonyl CoA and does not require ATP [9].

There was little or no stimulation of synthesis when $Mg^{++}$, $Mn^{++}$ or $HCO_3^-$ were included. A similar result was reported by Harlan and Wakil for rat liver preparations [14]. Coenzyme A exerted a considerable inhibitory effect at concentrations above 50 μM and slight stimulation below 25 μM (Fig. 1). Kanoh and Lindsay have reported inhibition at levels over 10 μM [16]. Coenzyme A is generally included in incubation systems at the levels used here in order to promote esterification of fatty acids with glycerol phosphate and thus remove them from the synthetase complex [17]. Because citrate (or isocitrate) activates acetyl CoA carboxylase in chain elongation [13], the promoting effect of isocitrate in the incorporation of acetyl CoA by the 15,000 $\times$ $g$ fraction (Table II) suggests the presence of a mitochondrial (but not microsomal) mechanism of this type with malonyl CoA as intermediate. However, the data of Table I indicate that malonyl CoA is a better substrate for the 100,000 $\times$ $g$ pellet fraction than acetyl CoA. It is possible that the carboxylase of this fraction is partially inactivated by the long centrifugation at low temperature. The exact mechanisms used by both subcellular fractions are under further study.

As also noted in other systems, anaerobiosis increases acetyl CoA incorporation [19,20]. According to Barron, this is probably due to avoidance of the oxidation of acetate to $CO_2$; this reaction causes depletion of the acetate pool [19].

Desaturase is a function of microsomal enzymes and requires oxygen [21]. Comparison of results obtained in oxygen and nitrogen should provide some information on the extent of desaturation [16]. Results showed that the distribution of radioactivity with nitrogen was very similar to that observed with oxygen although the overall radioactivity levels were increased (Table IV). This suggested that in the cell-free preparations unsaturated fatty acids of skin were labeled by chain elongation and not as the result of desaturation of a previously labeled acid. The principal reactions occurring were these elongations: 16:1 → 18:1 → 20:1, 18:2ω6 → 20:2ω6, and 20:4ω6 → 22:4ω6. Further evidence was obtained by measuring the proportion of radioactivity in carboxyl carbons (Table V).

The identification of polyunsaturated fatty acids was facilitated by previous studies on the polyenes of newborn mouse skin [2]. Most polyene GLC peaks represent a single isomer, which belongs either to the ω6 (linoleic) or ω3 (linolenic) family of polyunsaturated acids. Monounsaturated peaks represent mixtures of isomers. For example, 18:1 is

probably a mixture of ω9 (oleic acid) and other isomers which are formed probably by chain elongation of $C_{16}$ precursors. This was established in whole skin after incubation with [$^{14}$C]-acetate by the isolation of various radioactive dicarboxylic acids on oxidative degradation (unpublished data) of 18:1 material. Thus, in the absence of desaturase, the radioactivity associated with 18:1 represents the contributions of chain extension mechanisms. Retroconversion followed by chain elongation could also result in oleic labeling [25] but, as explained below, would not be expected to operate under conditions used here with subcellular preparations.

The data presented in Table VI suggest that in whole skin 18:2ω6 (linoleic acid) and other polyunsaturated acids are labeled by retroconversion followed by chain elongation using one [$^{14}$C]-acetate unit. In whole skin, linoleic acid is the most heavily labeled polyene [2], but is only slightly labeled (Table VI) in subcellular fractions using [1-$^{14}$C]-acetyl CoA. Retroconversion is the removal of acetate to give 16:2ω6 (in the case of linoleic acid). This mechanism has been demonstrated for $C_{22}$ polyenoic acids in rat liver mitochondria [23] and for $C_{18}$ acids (18:0, 18:1ω9) in rat liver microsomes [24]. The reaction is catalyzed by NAD and NADP and strongly inhibited by NADH and NADPH [26]. Thus, it would not occur under the experimental conditions used with subcellular fractions. However, we failed to observe this mechanism after incubation of mixtures containing the 15,000 × *g* or 100,000 × *g* pellets with the required cofactors [24], followed by addition of reduced cofactors and [1-$^{14}$C]-acetyl CoA for chain elongation. The demonstration of retroconversion using suitably labeled linoleic acid as its CoA derivative is under study.

Furthermore, the polyenes 18:3ω6 and 20:3ω6, both of which are usually synthesized from 18:2ω6 by desaturation, or desaturation plus chain elongation, possess 7.7 and 15.9% of the total polyene radioactivity in whole skin, but only 0.3 and 3.4% in experiments using the 15,000 × *g* pellet. It is possible that these two polyenes are labeled in whole skin by a retroconversion mechanism.

Further evidence of the existence of only low levels of desaturase activity in skin has been obtained in studies using labeled fatty acids (unpublished data). Further investigation is necessary to determine the origin or mode of biosynthesis of unsaturated acids such as oleic acid, 18:3ω6 and 20:3ω6 in skin.

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